PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PA132610/PCT	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/IB 03/05634	International filing date *(day/month/year)* 04.12.2003	Priority date *(day/month/year)* 04.12.2002
International Patent Classification (IPC) or both national classification and IPC C07K14/16		
Applicant UNIVERSITY OF CAPE TOWN et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
30.06.2004	09.03.2005
Name and address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Griesinger, I Telephone No. +49 89 2399-7596

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IB 03/05634

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-21 as originally filed

Sequence listings part of the description, Pages

22-27 as originally filed

Claims, Numbers

1-16 received on 21.02.2005 with letter of 21.02.2005

Drawings, Sheets

1/6-6/6 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☒ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☒ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

☐ the description, pages:

☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-11
	No:	Claims	12-16
Inventive step (IS)	Yes:	Claims	none
	No:	Claims	1-16
Industrial applicability (IA)	Yes:	Claims	1-16
	No:	Claims	none

2. Citations and explanations

see separate sheet

Re Item I

Basis for the report

1. The number of pages of the application documents as originally filed has been verified and corresponds to the page numbers indicated on the cover sheets. In particular, page 12 was filed with the original application documents.

2. The amended claims do not go beyond the disclosure of the application as originally filed.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Introduction

The following documents are referred to in the communication. The numbering will be adhered to for the rest of the procedure.

D1: WO 02 03917
D2: WO 00 39302
D3: Van Harmelen et al. 2001: Characterization of full-length HIV type 1 subtype C sequences from South Africa. AIDS research and human retroviruses Vol. 17, No. 16, pages 1527-1531.

The application relates to the production of HIV-1 gag protein, viral particles comprising said protein and vaccines comprising the protein or the particles. In the examples, the gag encoding sequence of the HIV-1 clade Du422 from South Africa is first codon optimized. Subsequently, the sequence is cloned either in a vector based on tobacco mosaic virus (TMV) and expressed in Nicotiana benthamiana plant cells or cloned in a baculovirus vector and expressed in insect cells. The sequence according to Seq. ID No. 2 is identical to the sequence according to Seq. ID No. 1 but 70 nucleotides shorter.

D1 discloses the cloning of the gag encoding sequence e.g. of the strain Du422 into an alphavirus such as equine encephalitis virus (VEE) using e.g. insect cells. The sequence of

the gag protein used in example 5 is 99,9% identical over the full length to the sequence according to Seq. ID No. 2 of the present application. However, the protein is modified to inhibit formation of virus-like particles.

D2 discloses the production of an HIV-1 gag polypeptide in mammalian, insect, or plant cells using e.g. viral vectors such as a baculovirus based vector. In order to increase the production of the protein, the HIV-1 codon usage pattern was modified.

D3 relates to the full-length subtype C sequences from four HIV-1 isolates from South Africa including the isolate Du422. The sequence is 99,4% identical to the sequence according to Seq. ID No. 1 over 1537 of 1549 nucleotides. The said gag encoding sequence is proposed to be used to produce a vaccine.

2. Novelty

The subject-matter of claims 12-16 does not fulfill the requirements of Article 33(2) PCT, since it is not novel in view of D3.

Claims 12 and 13 refer to virus-like particles, comprising the Pr55Gag protein according to Seq. ID Nos. 1 or 2 or a protein which is at least 90% identical to the sequence according to Seq. ID Nos. 1 or 2, wherein the sequence according to Seq. ID No. 2 is identical to the sequence according to Seq. ID No. 1 but 70 nucleotides shorter. Said virus-like particles are produced by expression of the coding sequence from a plant vector.

D3 already discloses a sequence which is 99,4% identical to the sequence according to Seq. ID No. 1 over 1537 of 1549 nucleotides. Said gag encoding sequence is proposed to be used to produce a vaccine e.g. by using the equine encephalitis virus VEE) replicon as a system. It seems that the expression of the gag coding sequences in VEE may result in the formation of virus-like particles.

Since it seems that the production of the virus-like particles using a plant vector does not change the structural features of the virus-like particles, the subject-matter of claims 12 and 13 is not considered to be novel in view of D3.

D3 also proposes the production of vaccines. Therefore, the subject-matter of claims 14-

16 is also not considered to be novel.

3. Inventive step

The subject-matter of all claims does not fulfill the requirements of Article 33(3) PCT, since it is not inventive.

Claims 1 and 2 refer to a plant vector comprising the gag encoding nucleic acid sequence of the HIV-1 clade Du422 from South Africa according to Seq. ID Nos. 1 and 2.

3.1 The subject-matter of claims 1 and 2 is not inventive when considering either D1 or D3 to be the closest prior art. Both documents already disclose the gag encoding nucleic acid sequence of the HIV-1 clade Du422 from South Africa and its use for producing a vaccine. The protein is expressed using e.g. a VEE vector and insect cells. Hence, the difference between D1 and D3 on the one side and the subject-matter of claims 1 and 2 on the other side resides in the use of a plant vector in claims 1 and 2. However, said difference can not make the subject-matter inventive, since the skilled practitioner in general considers different systems for the expression of a gene including a plant expression system. This is particularly true in view of D2, which already proposes the use of plant cells for the expression of said gene. Furthermore, the chosen system does not seem to have a surprising effect. In particular, the expression system does not necessarily result in the formation of virus-like particles, since the sequence with 90% identity may not allow the formation of virus-like particles (see e.g. example 5 of D1, where the 99,9% identical sequence does not allow the formation of virus-like particles). Consequently, the subject-matter of claims 1 and 2 is not inventive. Since the subject-matter of claims 3-6 is considered to be an arbitrary selection, said claims are also considered to be obvious.

3.2 The subject-matter of claims 1-16 is not considered to be inventive, when D2 is taken as the closest prior art.

D2 discloses the production of virus-like particles by expressing an HIV-1 gag polypeptide e.g. in plant cells (claim 44). In order to increase the production of the protein, the HIV-1 codon usage pattern is modified (page 42, line 1 to page 44, line 27). The subject-matter is exemplified by a baculoviral system using insect cells. D2 does not disclose the sequences of the gag peptide of the HIV-1 clade Du422.

Hence, the difference between D2 and the subject-matter claimed resides in the chosen variant of HIV-1 gag.

The objective problem can be formulated as the provision of virus-like particles of a different clade.

The solution, namely the plant vector comprising the sequences according to Seq. ID Nos. 1 or 2, is obvious in view of D2 in combination with either D1 or D3.

D1 and D3 respectively disclose the gag encoding nucleic acid sequence of the HIV-1 clade Du422 from South Africa and it's advantages for producing a vaccine (see e.g. D1: page 71, line 21 to page 72, line 7 and D3: page 1527, right-hand column, second paragraph to page 1528, left-hand column, first paragraph). Hence, the subject-matter of claims 1 and 2 seems to be a mere combination of the teaching of D2, namely to express HIV-1 gag in plant cells and the teaching of either D1 or D3, namely that the gag peptide of the HIV-1 clade Du422 is particularly useful. Consequently, the subject-matter of claims 1 and 2 is not considered to be inventive.

Since the use of the tobacco mosaic virus vector, of Agrobacterium tumefaciens, and of N. benthamiana are considered arbitrary selections, the subject-matter of claims 3-6 is also obvious.

The same considerations as for claims 1-6 apply for the expression of the protein in the form of virus-like particles (claims 7-11), the corresponding virus-like particles (claims 12 and 13) and the vaccines (claims 14-16).

3.3 In the application on page 9, second paragraph, is stated that it was not predictable whether virus-like particles comprising HIV-1 gag would assemble correctly in plant and insect cells. This argument is supported by prior art documents which show that virus-like particle assembly is sensitive to the chosen cell type and that in some systems viral-like particles are not or only inefficiently formed. Hence, the question arises, if the proposed use of plant cells for the production of virus-like HIV particles in D2 is sufficient to motivate and enable the person skilled in the art to produce said virus-like particles. In general, the skilled practitioner assumes that a given gene can be expressed in numerous expression

systems, wherein the choice of the expression system depends on well known advantages or disadvantages of said systems. Hence, as long as there is no clear indication that an expression system does not work, the skilled practitioner would consider said expression system. This is also true for the specific protein HIV p55 gag. According to the application, the outcome of the expression of HIV p55 gag in insect cells was unpredictable. However, D2 shows in example 5 that HIV p55 gag can be expressed in insect cells and that virus-like particles are formed. In view of said example, the skilled practitioner had no reason to doubt that the other expression systems proposed in D2 would work. Hence, the skilled practitioner was motivated to try to express HIV-1 gag in plant cells. He also had a reasonable expectation of success, since D2 provides sufficient guidance about the cloning procedure and necessary adaptations to the respective cloning system. For example, it was proposed to modify the coding sequence to reflect the codon usage of the host cell (page 42, line 1 to page 44, line 27).

3.4 In general, the same requirements for sufficiency of disclosure have to be met by the prior art and the application. Hence, if the assembly of virus-like particles in one expression system does not at all allow to predict the outcome for a different expression system, the generalization in the present application from the expression system using the tobacco mosaic virus in cells of Nicotiana benthamiana to any plant vector in any plant may also not be allowable. In this case, the subject-matter of claims 1 and 2 would be regarded to be broader than justified by the description, i.e. to lack support by the description (Article 6 PCT). However, in this case, a claim restricted to the production of virus-like particles in Nicotiana benthamiana may be considered to be novel and inventive.

4. Further observations

The document D4 (WO 03 004620) has not been considered in the present first communication, since it was published after the priority date of the present patent application and regulations concerning such "PX" documents differ between the PCT member states. However, the document may be relevant for accessing novelty and inventive step of the present application.

CLAIMS

1. A plant vector including a nucleotide sequence encoding an HIV Gag polypeptide, wherein the nucleotide sequence encoding the Gag polypeptide comprises a sequence having at least 90% sequence identity to the sequence set forth in SEQ ID NO: 1.

2. A plant vector including a nucleotide sequence encoding an HIV Gag polypeptide, wherein the nucleotide sequence encoding the Gag polypeptide comprises a sequence having at least 90% homology to the sequence set forth in SEQ ID NO: 2.

3. A vector according to either of claims 1 or 2, which is a tobacco mosaic virus vector.

4. A vector according to either of claims 1 or 2, which is an *Agrobacterium tumefaciens* containing a T-derived plasmid construct.

5. A plant cell including a vector according to any one of claims 1 to 4, wherein the nucleotide sequence is operably linked to control elements compatible with expression in the cell.

6. A cell according to claim 5, which is a *N. benthamiana* plant cell.

7. A method of producing an HIV-1 immunogenic protein or a related polypeptide which is assembled into the form of virus-like particles, the method comprising the steps of:

(a) introducing a plant vector or vector system into a host plant cell, the vector or vector system including a nucleic acid sequence encoding the HIV-1 immunogenic protein or related polypeptide derived by substitution, deletion and/or insertion of one or more nucleotides, and/or extension or truncation of one or both ends thereof, the nucleic acid sequence having at least 90% identity to the sequence set forth in SEQ ID NO:1;

(b) causing expression of the nucleic acid sequence in the host cell; and

(c) recovering the resulting HIV-1 immunogenic protein virus-like particles or related polypeptide virus-like particles produced within the host cell.

8. A method of producing an HIV-1 immunogenic protein or a related polypeptide which is assembled into the form of virus-like particles, the method comprising the steps of:

(a) introducing a plant vector or vector system into a host plant cell, the vector or vector system including a nucleic acid sequence encoding the HIV-1 immunogenic protein or related polypeptide derived by substitution, deletion and/or insertion of one or more nucleotides, and/or extension or truncation of one or both ends thereof, the nucleic acid sequence having at least 90% identity to the sequence set forth in SEQ ID NO:2;

(b) causing expression of the nucleic acid sequence in the host cell; and

(c) recovering the resulting HIV-1 immunogenic protein virus-like particles or related polypeptide virus-like particles produced within the host cell.

9. A method according to either of claims 7 or 8, wherein the vector is a tobacco mosaic virus vector.

10. A method according to either of claims 7 or 8, wherein the vector is an *Agrobacterium tumefaciens* containing a T-derived plasmid construct.

11. A method according to any one of claims 7 to 10, wherein the plant cell is a *N. benthamiana* plant cell.

12. An HIV-1 protein or polypeptide that is produced according to the method of any one of claims to 7 to 11, and which is assembled into the form of virus-like particles.

13. A protein or polypeptide according to claim 12, which is an HIV-1 Pr55 Gag protein.

14. A vaccine for use in the treatment or prophylaxis of HIV infection in a mammal, the vaccine including virus-like particles of proteins or polypeptides as described in either one of claims 12 or 13.

15. A vaccine according to claim 14, which induces an immunogenic response to the virus-like particles in a suitable susceptible host.

16. A vaccine according to either one of claims 14 or 15, which includes a pharmaceutical excipient and/or adjuvant, and a therapeutically effective amount of the virus-like particles.